

Modulating the Autoimmune Response in Type 1 Diabetes:

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■ Abstract

Type 1 diabetes mellitus results from a loss of insulin-producing β -cells in the pancreatic islets caused by an immune-mediated chronic destructive process. It is generally believed that immune tolerance to β -cells is broken by environmental factors in genetically susceptible individuals, leading to β -cell destruction that is mediated by T lymphocytes. A key assumption in the current pathogenic concept of type 1 diabetes is a defective immunoregulation affecting both central and peripheral mechanisms of tolerance induction against β -cell antigens. In animal models of type 1 diabetes, disease-protective modulation of the islet autoimmune response can be effected by various strategies including

administration of islet antigens. In human type 1 diabetes, therefore, new strategies are currently being developed with the aim of actively suppressing the autoimmune process and inducing a lasting tolerance against islet antigens. In this context, inducing regulatory T cells *in vivo* (i.e. CD4⁺CD25⁺ T cells or type 1 regulatory T cells) is currently becoming more widespread. The following report highlights some of the recent studies on immunotherapy of type 1 diabetes, presented at the 64th Scientific Sessions, held in June 2004, in Orlando, Florida.

Keywords: immunomodulation · immunotherapy · tolerance · Treg cells · type 1 diabetes

Modulation of the immune response in newly diagnosed patients with type 1 diabetes through NBI-6024, an altered peptide-ligand of the immunodominant insulin epitope B9-23

NBI-6024 corresponds to a peptide of the B-chain of insulin (B9-23) representing one of the target epitopes recognized by autoreactive, IFN- γ producing T lymphocytes. Administration of the altered peptide-ligand NBI-6024 is supposed to exert an immunomodulatory effect on autoaggressive T cells and is currently being tested in a phase II worldwide multicenter trial. David Alleva presented work in which T cells from 40 patients from a completed NBI-6024 phase I trial were isolated and tested for IFN- γ secre-

tion in the presence of insulin B9-23 or NBI-6024 using ELISPOT-assays [1]. After stimulation with insulin B9-23, IFN- γ secretion was observed in 50% of patients treated with placebo, compared to 8% of the non-diabetic control population. Interestingly, only 6% of the adolescent, NBI-6024-treated patients showed a B9-23-stimulated IFN- γ response while T cells from 75% of these patients showed a significant IL-5 secretion suggesting a Th2-like immune response. Compared to adolescents, NBI-6024 treated adult patients with newly diagnosed type 1 diabetes more often had a B9-23 stimulated IFN- γ secretion (31%). However, this IFN- γ response could be fully suppressed by increasing the dosage of NBI-6024 (50% of patients with IFN- γ secretion after administration of 0.1 mg NBI-6024; 38% by 1 mg; and 0% by 5 mg NBI-6024; ap-

plied subcutaneously 5 times over a period of 8-12 weeks).

This study showed that NBI-6024 dose-dependently suppresses IFN- γ secretion by autoaggressive Th1 cells. The observed increase of IL-5 secretion in the presence of insulin B9-23 is suggestive of a modulation of the immune response towards a protective Th2 response against islet antigens. Although numbers of patients treated with NBI-6024 are still small, these results are encouraging with respect to the ongoing phase II trial in newly diagnosed patients with type 1 diabetes aiming to preserve residual C-peptide.

The PREVEFIN Study: primary prevention with Vitamin D and cow's milk hydrolysate

Early exposure to the cow's milk antigen β -casein has been discussed as a possible trigger for the initiation of human islet autoimmunity. It has also been proposed that lack of Vitamin D favors the development of autoimmunity, since active Vitamin D3 has immunoregulatory properties and promotes maturation of the small bowel mucosa thus regulating absorption of high-molecular weight proteins. Renata Lorini [2] presented preliminary results of the PREVEFIN study. In this study, newborns from the general population from 11 centers in Italy were screened for HLA risk alleles associated with type 1 diabetes. Children genetically at high risk for the disease were randomized into two intervention groups and followed for the development of islet autoantibodies. During the first 12 months after birth, children allocated to group A received 500 IU Vitamin D/day plus a diet with cow's milk hydrolysate. Children from group B received 500 IU Vitamin D/day added to a non-restricted diet. To date, 9909 newborns have been screened. Of these, 73 children had a high risk HLA-haplotype, and 31 and 27 children were randomized to groups A and B, respectively. Sixteen children have dropped out of the study, and 42 children have been closely followed with visits every 3 months. So far, four children have developed islet autoantibodies, three in group A (GAD autoantibodies), and one in group B (GAD and transient insulin autoantibodies).

Interestingly, all three children from group A have developed antibodies against GAD, but not to insulin, which is in contrast to the findings of prospective studies following children from birth for the development of islet autoimmunity. Usually, early childhood islet autoimmunity is associated with the development

of insulin autoantibodies (IAA) being present in almost 100% of children affected with islet autoimmunity during the first two years of life [3]. The lack of IAA in the three children from intervention group A could, therefore, represent an immunomodulatory effect of the combined intervention strategy. Further follow-up will show whether the GAD autoantibody-positive individuals will progress to multiple islet autoantibodies including IAA, which would be expected from the natural course of islet autoimmunity in young children.

Treatment with hOKT3 γ 1 improves insulin response and reduces insulin requirements in patients with new onset type 1 diabetes

Kevin C. Herold presented data from a randomized, placebo-controlled multicenter trial that tested the effects of a treatment with the monoclonal anti-CD3 antibody hOKT3 γ 1 (Ala-Ala) on β -cell reserve in newly diagnosed patients with type 1 diabetes [4]. Ten patients were included in the study (six patients were treated with anti-CD3 antibody, 4 patients were given placebo). The antibody was administered intravenously on 12 consecutive days. The number of circulating lymphocytes after treatment was reduced to 17% of baseline levels but recovered to 108% by 6 weeks after the last injection of the anti-CD3 antibody. Mixed-meal-stimulated C-peptide AUC (area under the curve) levels were measured at study entry and after 6 and 12 months, respectively. At entry, mean levels of stimulated C-peptide were similar between verum and placebo-treated patients; however, after 12 months, fasting C-peptide levels were reduced in control patients by 68% vs. only 1.4% in antibody-treated patients ($p < 0.01$). Correspondingly, stimulated AUC C-peptide levels were significantly higher in antibody-treated individuals (10.7 ng/ml/4h) compared to placebo-treated patients (2.3 ng/ml/4h). Mean HbA1c values did not differ after one year (6.8% in the verum- vs. 7.0% in the control-group), whereas antibody-treated patients had lower insulin requirements (0.33 U/kg) compared to placebo-treated patients (0.68 U/kg, $p < 0.01$). Frequencies of antibodies to GAD, IA-2 and insulin were not changed by the treatment with hOKT3 γ 1.

This study confirmed the protective effect on β -cell reserve in newly diagnosed patients with type 1 diabetes by treatment with one course of monoclonal anti-CD3 antibody. However, compared to the pilot study [5], in this second trial, a four-fold higher dose of hOKT3 γ 1 was used which was associated with more

pronounced adverse effects including one patient who recovered from lymphopenia only slowly. It will be interesting to see whether the markedly protective effect on β -cell reserve will last on follow-up.

Modulation of the autoimmune process in LADA patients by treatment with recombinant GAD65 is safe and does not impair β -cell function

Modified human recombinant GAD65 (Diamyd™) was tested in a randomized, double-blind, placebo-controlled study in 47 patients with latent autoimmune diabetes (LADA) to show that Diamyd is safe and does not impair the function of β -cells [6]. Participants in the study, presented by Carl-David Agardh, received either placebo or a subcutaneous injection of 4, 20, 100 or 500 μ g Diamyd, respectively, after one and 4 weeks, and were followed over a period of 24 weeks. None of the patients showed study-associated adverse effects. After 24 weeks, patients who received 20 μ g Diamyd had significantly higher levels of fasting C-peptide compared with placebo-treated patients. In this intervention group, both median levels of fasting C-peptide and mixed-meal-stimulated C-peptide levels increased by 36% and 19%, respectively, compared with baseline levels. Patients treated with the highest dose of Diamyd (500 μ g) showed a significant increase of antibodies to GAD. An increase in CD4⁺CD25⁺ regulatory/CD4⁺CD25⁻ T-cell ratio was positively correlated with a change in fasting ($r = 0.56$, $p < 0.001$) and stimulated ($r = 35$, $p = 0.04$) C-peptide levels over 24 weeks.

This study is the first to show that induction of putatively regulatory T cells can be effected in humans by treatment with autoantigen. However, the results are preliminary and factors that are known to influence the risk of progression to insulin dependency in LADA patients such as GADA titer and age have to be considered.

Immunomodulatory effect of Glucagon-like peptide-1 (GLP-1)

Kai Masur reported, for the first time, an immunomodulatory effect of GLP-1 [7]. Human T lymphocytes express GLP-1 receptors, and physiologic GLP-1 levels stimulate migration of T-cells. In contrast, high levels of GLP-1 impair the mobility of CD8⁺ T-cells (homing effect). Interestingly, a further report by the authors showed that pancreatic β -cells also produce

biologically active GLP-1 in physiologically relevant amounts [8]. Based on these findings, it has been postulated that GLP-1 released from intestinal L-cells may have a role in the immune response against microbial agents in the gut by functioning as a slow-down signal for CD8⁺ cells thus prompting the cells to home into the intestine during food intake to fend off invading microorganisms. However, in patients with type 1 diabetes, one could speculate that GLP-1 could be released either from damaged β -cells or from precursor β -cells as a consequence of increased β -cell neogenesis that results in a potentially negative homing effect on autoreactive CD8⁺ T cells contributing to β -cell destruction. These results support the view that GLP-1 may play a role in the pathophysiology of type 1 diabetes.

CD11a antagonist cLABL inhibits adhesion of T lymphocytes to pancreatic islet microvascular endothelium

Adhesion molecules like the leukocyte function associated antigen-1 (LFA-1, CD11a) may be important for recruitment of islet-infiltrating autoimmune T cells in the course of islet autoimmunity. Therefore, blocking interactions between CD11a and its respective ligand on the surface of endothelial cells could inhibit T cell migration into the pancreas and therefore have a protective effect on the development of insulinitis and autoimmune diabetes. Using a real-time *in vitro* hydrodynamic flow chamber model, Christopher G. Kevil [9] was able to demonstrate that adhesion of a CD11a⁺ T cell line (WEHI 7.1) to microvascular endothelial cells of pancreatic islets was increased by administration of TNF- α through up-regulation of ICAM-1 and VCAM-1 but decreased by giving the cyclic anti-CD11a peptide-antagonist cLABL. These results suggest that CD11a antagonists may have a potential value for the treatment of type 1 diabetes.

Concluding remarks

Using sophisticated measurements of islet autoantibodies we are now able to predict type 1 diabetes [10, 11], and trace its natural history [3]. We have little idea, however, of the etiologic mechanisms that trigger autoimmunity and promote progression to disease, nor do we have ready access to autoreactive T-cells within the pancreas that are putatively responsible for disease or the possibility to quantify and characterize these cells. Advances in these areas are necessary if we are to

fully understand the autoimmune pathogenesis of type 1 diabetes. However, we now have the ability to apply new therapeutic strategies aiming to halt or prevent β -

cell destruction to carefully selected individuals who might profit most from immune intervention.

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